Foliar Chemistry Linked to Infestation and Susceptibility to Hemlock Woolly Adelgid (Homoptera: Adelgidae)

JENNIFER A. PONTIUS,[1] RICHARD A. HALLETT, AND JENNIFER C. JENKINS[2]

USDA Forest Service–Northeastern Research Station, 271 Mast Road, Durham, NH 03024

ABSTRACT   Hemlock woolly adelgid (*Adelges tsugae* Annand) is an invasive insect pest that is causing widespread mortality of eastern hemlock. However, some stands remain living more than a decade after infestation. To date, this has been attributed to site and climatic variables. This multi-tiered study examines the role foliar chemistry may play in *A. tsugae* success and subsequent hemlock decline. Comparisons of resistant and susceptible hemlock species indicate higher concentrations of P and lower concentrations of N in resistant species. On experimentally colonized hemlocks, the numbers of live sistens present after two *A. tsugae* generations was correlated with higher K and lower P concentrations. A regional *T. canadensis* monitoring effort showed that concentrations of Ca, K, N, and P were most strongly correlated with *A. tsugae* densities, which was the driving factor in hemlock decline. From the results of this study, we hypothesize that higher N and K concentrations may enhance hemlock palatability, thereby increasing *A. tsugae* population levels, whereas higher concentrations of Ca and P may deter more severe infestations. Foliar chemistry alone can explain over one-half of the variability in hemlock decline witnessed at 45 monitoring plots across the northeastern United States. Combining chemistry and traditional site factors, an 11-class decline rating could be predicted with 98% 1-class tolerance accuracy on an independent validation set. These results suggest that foliar chemistry may play a role in eastern hemlock susceptibility to *A. tsugae* infestation and should be included in risk assessment models.

KEY WORDS   exotic pest, forest health, eastern hemlock, *Tsuga canadensis*

SINCE THE 1980s, WHEN the hemlock woolly adelgid (*Adelges tsugae* Annand) was introduced to the northeastern United States, it has spread rapidly, leading to eastern hemlock (*Tsuga canadensis* Carriere) decline and mortality from North Carolina to Massachusetts (Souto et al. 1995). Most infested hemlock have shown no resistance to *A. tsugae* and little chance for recovery (McClure 1995).

Among hemlock species, *Tsuga diversifolia* Masters (northern Japanese hemlock), *T. chinensis* Franch. (Chinese hemlock), and *T. sieboldii* Carriere (southern Japanese hemlock) have experienced minimal impact to hemlock populations. This has been attributed to a combination of natural predators and host resistance (McClure 1995). In the western United States, *T. heterophylla* Sargent (western hemlock) and *T. mertensiana* Carriere (mountain hemlock) also show low mortality after infestation (McClure 1992). The mechanisms behind this host resistance remain unclear.

There is evidence that the impact of *A. tsugae* on eastern hemlock varies significantly with site conditions and the presence of other stressors, resulting in adjacent stands responding differently to attack (Orwig and Foster 1998, Sivaramakrishnan and Berlyn 1999). We hypothesize that a relationship exists between foliar nutrient status, *A. tsugae* infestation, and hemlock decline. For example, it is understood that herbivory is usually positively correlated with foliar nitrogen concentrations and that low nitrogen concentrations in foliage can limit insect populations (McClure 1980, White 1984, Schowalter et al. 1986). In eastern hemlock, fertilization resulted in increased relative growth rate, survivorship, and fecundity of *A. tsugae* (McClure 1991, 1992). McClure (1992) also found that nitrogen fertilization increased eastern hemlock susceptibility to *A. tsugae* and reduced the effectiveness of implanted and injected pesticides. However, the role of other essential macronutrients in *A. tsugae* population patterns has not been examined. Of particular interest are Ca and P, which have been implicated in suppressing aphid populations on agricultural crops (Chhillar and Verma 1985, Harada et al. 1996).

If there are significant differences in foliar chemistry between resistant and susceptible species, and

[1] Corresponding author: USDA Forest Service, NE Research Station, 271 Mast Road, Durham, NH 03024 (e-mail: jpontius@fs.fed.us).
[2] Gund Institute for Ecological Economics, University of Vermont, 590 Main Street, Burlington, VT 05405–0088.

Table 1. Statistical methods

Task	Analysis	df	P limit	Reference
Species comparison				
Arboretum resistant versus susceptible	Student's t-test	17	0.01	Zar 1984
Database resistant versus susceptible	Student's t-test	98	0.01	Zar 1984
Colonization study				
Infestation versus chemistry	Spearman's rho rank correlations	16	0.10	Pagano and Gauvreau 2000
Predictive models	Stepwise mixed linear regression	16	0.10	Pagano and Gauvreau 2000
Species effect	ANOVA	16	0.10	Selvin 1995
Regional study				
Stand characteristics	Student's t-test	44	0.01	Zar 1984
Foliar chemistry	Pairwise correlations	225	0.01	Zar 1984
	Partial correlations	225	0.01	Selvin 1995
Predictive models	Stepwise mixed linear regression	219	0.01	Pagano and Gauvreau 2000

Three tiers of this study required different statistical tests depending on the design and nature of the data. Here we summarize the statistical methods used for the various components of this study.

these same elements are strongly correlated with colonization success, we hypothesize that such elements may play a role in host susceptibility. If these same relationships are maintained for eastern hemlock across our regional study area, foliar chemistry may also be used to help identify stands relatively less susceptible to *A. tsugae* in the Northeast.

To more fully understand the relationship between foliar chemistry, *A. tsugae,* and hemlock decline, this study took a multi-tiered approach, with interspecies comparisons, artificial colonization, and regional studies. The specific objectives were to (1) determine if foliar cations in *A. tsugae* resistant hemlock species (*T. chinensis, T. diversifolia, T. heterophylla, T. mertensiana,* and *T. sieboldii*) differ significantly from *T. canadensis,* (2) determine if foliar cations are significantly correlated with colonization success on *T. canadensis, T. chinensis, T. diversifolia, T. heterophylla,* and *T. sieboldii*, and (3) examine relationships among foliar cation concentrations, *A. tsugae* population density, and severity of *T. canadensis* decline on a regional scale.

Materials and Methods

Interspecies Comparison. At the Arnold Arboretum in Jamaica Plain, MA, foliage was collected from 18 hemlocks including the resistant *T. diversifolia* ($n = 4$), *T. sieboldii* ($n = 2$), *T. heterophylla* ($n = 4$), and *T. chinensis* ($n = 2$) and the susceptible *T. canadensis* ($n = 6$). All trees were mature, healthy landscape specimens, growing on similar soil type and under similar light, climate, and growth conditions. Hand shears were used to collect terminal branches from multiple locations on each study tree.

Needles were dried at 70°C and ground to pass a 1-mm mesh screen. A NIRSystems spectrophotometer (NIRSystems Inc., Silver Spring, MD) was used to measure N concentrations (Bolster et al. 1996). Dried and ground foliage was digested using a microwave assisted acid digestion procedure (EPA method 3052) and analyzed for Ca, K, Mg, Mn, and P using a Varian axial inductively coupled plasma spectrometer (Varian Inc., Walnut Creek, CA).

Foliar N and P data were also obtained for an additional 50 *T. heterophylla* and 5 *T. mertensiana* collected in the Pacific Northwest and archived at Oak Ridge National Laboratory Distributed Active Archive Center (Matson 1994). These were compared with 44 eastern hemlock samples from the northeastern Northeastern Ecosystem Research Cooperative foliar chemistry database (Northeastern Ecosystem Research Cooperative 2003). Student's t-tests were used to determine if significant differences existed between resistant and susceptible species (Table 1).

Colonization Study. The uninfested Arnold Arboretum trees described above were artificially infested by attaching heavily *A. tsugae*–infested *T. canadensis* branches to three healthy branches of each intended host. In this way, new crawlers from the infested branch could easily crawl and attach to the intended host tree. To prevent further spread, *A. tsugae* were contained on target host branches for the duration of the study with mesh bags. After 6 mo, bagged branches were collected, and the number of live sistens on the recipient host branch was recorded. Foliar Ca, K, Mg, Mn, N, and P were determined for preinfestation (collected coincident with attachment of *A. tsugae*–infested branches) and postinfestation (bagged samples collected at the end of the colonization study) using the methods described above. The entire bagged sample was examined, and the total numbers of live *A. tsugae* sistens were recorded. Because of the non-normal distribution of the data, Spearman's rho rank correlations (Pagano and Gauvreau 2000) were used to identify significant relationships between chemistry and live sisten counts. A linear regression based on significant variables was constructed to determine the total variability in colonization success that could be accounted for by foliar chemistry (Kleinbaum et al. 1998) (Table 1).

Regional Study. In 2001, 45 *A. tsugae* monitoring plots (20 by 20 m) were established with the assistance of federal, state, and private land managers in Connecticut, Massachusetts, New Hampshire, New Jersey, New York, and Pennsylvania. These were selected to represent the range of infestation levels, infestation

Table 2. Summary of all the measured variables from the 45 northeastern hemlock monitoring plots

Variable	Uninfested		Infested	
	Mean (SD)	Range	Mean (SD)	Range
HWA and health parameters				
Percent infestation	0 (0)	0–0	36 (2.85)	1–100
Health rating	2.26 (0.07)	0.63–4.63	3.55 (0.11)	0.75–7.25
Years infested	0 (0)	0–0	5 (0.31)	1–11
Percent new growth	81 (1.80)	3–100	62 (2.56)	2–100
Percent transparency	12 (0.51)	4–42	19 (0.81)	4–67
Percent fine twig dieback	4 (0.29)	0–15	8 (0.63)	0–35
Percent living crown	61 (1.21)	31–100	57 (1.23)	16–93
Foliar chemistry				
Al (mg/kg)	309 (10.73)	91–752	451 (12.81)	111–870
Ca (mg/kg)	5038 (170.03)	1879–9036	6063 (244.95)	2416–16369
Fe (mg/kg)	51 (1.87)	32–167	61 (1.27)	40–154
K (mg/kg)	6837 (82.97)	5094–9036	7420 (108.18)	4405–10614
Mg (mg/kg)	1265 (25.16)	735–1829	1275 (37.28)	622–3583
Mn (mg/kg)	828 (45.85)	129–2360	1434 (74.88)	94–3699
P (mg/kg)	1476 (38.38)	723–2762	1786 (45.59)	923–3910
Sr (mg/kg)	9 (0.60)	1–31	11 (0.73)	3–60
N (%)	1.46 (0.01)	1.20–1.82	1.54 (0.02)	1.12–1.94
Lignin (%)	17.00 (0.11)	14.84–19.47	17.33 (0.11)	14.33–19.84
Cellulose (%)	31.00 (0.21)	23.66–35.25	31.53 (0.17)	26.81–36.00
Stand characteristics				
Total BA (m²/ha)	758 (14.78)	365–1150	658 (10.72)	365–920
Percent EH	73 (1.20)	45–93	72 (1.01)	48–95
Percent dead EH	7 (0.56)	0–19	12 (1.28)	0–55
Mean EH BA (m²/ha)	21 (0.54)	13–35	23 (0.73)	14–53
Elevation (m)	329 (21.56)	26–679	237 (12.90)	102–1710
Mean EH height (m)	26 (0.43)	17–39	28 (0.45)	16–41

histories, hemlock decline symptoms, and site nutrient status found across the northeastern United States.

On these plots, hemlock ranged from 45 to 95% of the total basal area and was similar between infested and uninfested stands. Selected plots were homogeneous, mature stands with a mean hemlock basal area of 21 and 23 m^2/ha for uninfested and infested stands, respectively (Table 2). In addition to eastern hemlock, the most commonly occurring species included red maple (*Acer rubrum* L.), yellow birch (*Betula alleghaniensis* Britt.), black birch (*Betula lenta* L.), and American beech (*Fagus grandifolia* Ehrh.).

The average decline rating for infested trees was 3.5. This was significantly higher than the 2.3 rating for uninfested trees (Table 2). Percent infestation ranged from 0 to 100, with an average of 36% on infested plots. Infested plots ranged from 0 to 55% dead hemlock, with an average of 12% dead standing basal area. This was significantly more than the 7% average for uninfested plots.

Within each plot, five dominant or co-dominant hemlock trees were selected for foliage sampling and decline assessments. Diameter at breast height (dbh) and species was recorded for all trees with >5 cm dbh within each plot. Foliage was collected from the mid and upper canopies of each tree using a 12-gauge shotgun. This sampling technique allowed us to efficiently target multiple sun-lit branches for collection from mature trees that are otherwise inaccessible. On falling to the ground, foliage was combined and immediately sealed, refrigerated, and transported back to the laboratory for analysis. From the collected sample, between 100 and 150 terminal branchlets were inspected for the presence of early-instar *A. tsugae*

using a ×10 hand lens. Infestation levels are reported as a percentage of terminal branches infested. Similarly, we assessed the percentage of terminal branchlets producing new growth. Foliar chemistry was quantified using the methods described above.

Hemlock decline was assessed using methods specifically designed to quantify the various, sequential symptoms that follow *A. tsugae* infestation. This included the percent of terminal branchlets with new growth, percent transparency (quantified using a concave spherical densiometer) (Pontius et al. 2002), percent fine twig dieback, and live crown ratio (USDA Forest Service 1997). The categories of hemlock health described in Table 3 reflect the typical characteristics for each measured variable at various stages of hemlock decline. Measured data were normalized to a category value according to Table 3 and averaged to determine one summary decline rating that best described overall health (a continuous variable where 0 = perfect health and 10 = dead).

Pairwise multiple correlations were used to examine relationships between hemlock decline ratings and foliar concentrations (Zar 1984). Because of the non-normal distribution of the percent infestation data, Spearman's rho rank significance tests were used for correlation analyses (Pagano and Gauvreau 2000). Because many of the chemical variables included in this study were highly correlated with each other, partial correlations based on significant pairwise variables were included to isolate relationships with infestation and decline and remove the effects of multi-collinearity between elements (Selvin 1995) (Table 1).

To determine how much variability in decline could be explained by chemistry, mixed-stepwise linear re-

Table 3. Decline classes

Category	Health status	Characteristics
0	Perfect health	100% new growth Less than 5% canopy transparency No fine twig dieback Greater than 90% photosynthetically active canopy
1	Very healthy	Over 90% new growth 6–9% canopy transparency Negligible fine twig dieback 80 to 89% photosynthetically active canopy
2	Healthy (typical forest co-dominant)	Over 85% of branches produce new growth 10–14% canopy transparency Less than 5% fine twig dieback 70–79% photosynthetically active canopy
3	Earliest decline	80–84% of branches produce new growth 15–19% canopy transparency 10% fine twig dieback 65–69% photosynthetically active canopy
4	Light decline	75–79% of branches produce new growth 20–24% canopy transparency Approaching 10% fine twig dieback 60–64% photosynthetically active canopy
5	Light to moderate decline	70–74% of branches produce new growth 25–29% canopy transparency 10–15% fine twig dieback 50–59% photosynthetically active canopy
6	Moderate decline	60–69% of branches produce new growth 30–34% canopy transparency Up to 15% fine twig dieback 40–49% photosynthetically active canopy
7	Moderate to severe decline	40–59% of branches produce new growth 35–39% canopy transparency 15–20% fine twig dieback 30–39% photosynthetically active canopy
8	Severe decline	20–39% of branches produce new growth 40–44% canopy transparency Up to 20% fine twig dieback 20–29% photosynthetically active canopy
9	Death imminent	Less than 20% of branches produce new growth Greater than 45% canopy transparency Greater than 25% fine twig dieback Less than 20% photosynthetically active canopy
10	Dead	100% Defoliated

Raw measured health variables were converted to a 0–10 scale before averaging to calculate the overall decline summary value for each tree. Category assignments are summarized here for new growth, transparency, dieback, and percent live crown and represent the typical charcteristics for each decline class.

gressions were constructed including site variables only, chemistry variables only, and full combined models on a 180-sample calibration set (Pagano and Gauvreau 2000). Variables were retained in the model if the P value was <0.01 and the variance inflation factor was <2.0. Potential models were compared based on Mallow's Cp and PRESS statistics (Kleinbaum et al. 1998) (Table 1). The final models were predicted on a 45-sample independent validation set to assess overall accuracy.

Results and Discussion

A complex suite of biological, chemical, and environmental variables governs the suitability of a host tree. Several studies (Bonneau et al. 1997, Royle and Lathrop 1999, Mayer et al. 2002, Orwig et al. 2002) have shown that site and climatic factors, such as winter temperatures, landscape position, and aspect, may influence hemlock decline on *A. tsugae*–infested trees. However, based on the results presented here,

we suggest that the inclusion of chemical factors is essential in understanding regional infestation and decline patterns.

If foliar chemistry plays a part in resistance, one would expect to see inherent differences in foliar chemistry between resistant and susceptible species. Furthermore, we hypothesized that *A. tsugae* would be unable to establish a viable population on poor chemistry host trees, regardless of species. If these same relationships are evident within eastern hemlock, foliar chemistry may play a role in relative susceptibility within the eastern hemlock population. While multiple cations were measured, only Ca, N, K, and P were consistently significant in all parts of this study and will be discussed further.

Nitrogen. Comparisons of new growth foliage from various species at the Arnold Arboretum indicated that *T. canadensis* had significantly higher N than all other resistant species (*P = 0.01*; Table 4). Comparisons of the western and eastern North American species collected within their native habitats showed that

Table 4. Species comparisons

	Variable	Resistant mean ± SE	Susceptible mean ± SE	t-test	P
Arnold Arboretum	Ca	2844 ± 651	1989 ± 771	−0.847	0.42
	K	8920 ± 911	11410 ± 1077	1.764	0.11
	Mg	1116 ± 127	1216 ± 151	0.504	0.63
	Mn	304 ± 58	328 ± 68	0.269	0.79
	N	1.92 ± 0.13	2.59 ± 0.15	3.265	0.01
	P	3270 ± 438	4318 ± 518	1.542	0.15
Database comparison	N	1.14 ± 0.02	1.24 ± 0.03	2.66	0.01
	P	1496 ± 42	1087 ± 50	−6.21	<0.0001

Comparison of various hemlock species showed significant differences in N and P concentrations between resistant and susceptible hemlock species. This was true for specimens grown under controlled conditions at the Arnold Arboretum and from native, in situ collections contained in online foliar chemistry databases.

the resistant western species also had significantly lower N than *T. canadensis* ($P < 0.0001$; Table 4). The fact that resistant species all have significantly lower N than eastern hemlock suggests that there could be some benefit to having lower N. The regional data further support this hypothesis. While percent infestation was positively correlated with foliar Ca, K, Mn, N, and P, partial correlations indicated that N had the strongest relationship with infestation (Table 5), with higher N consistently associated with higher *A. tsugae* population levels.

There is a large body of evidence to support a palatability-based hypothesis for N. Nitrogen has been shown to be positively associated with aphid success for a variety of host species (Carrow and Betts 1973, McClure 1980, Koritsas and Garsed 1985, Douglas 1993). Specific to eastern hemlock, N fertilization resulted in increased relative growth rate, survivorship, and fecundity of *A. tsugae* (McClure 1991, McClure 1992). McClure (1992) also found that nitrogen fertilization increased eastern hemlock susceptibility to *A. tsugae* and reduced the effectiveness of implanted and injected pesticides. This suggests that inherently low N concentration may limit *A. tsugae* success, which in turn may impart some measure of resistance

for host trees. Nitrogen is particularly important to insects because there is a large difference between the nitrogen concentration of plants (around 2% dry weight) and that of insects (approaching 7%) (Dale 1988). For relatively immobile insects, such as *A. tsugae*, the nutritive quality of forage becomes even more important. Under low nitrogen conditions, concentrations may not be sufficient to maintain viable *A. tsugae* populations. We conclude that higher foliar N concentrations may lead to higher *A. tsugae* infestation levels because of the increased nutritive value of the foliage. This increased *A. tsugae* population leads to more severe hemlock decline symptoms. This is shown in the regional study where foliar transparency and fine twig dieback are associated with higher foliar N concentrations (Table 5). Based on these findings, it is possible that eastern hemlock stands with relatively higher N nutrient status may be predisposed to catastrophic infestations, ultimately leading to their demise. This may be most common in mixed stands with higher quality litter inputs from deciduous species, or in areas approaching N saturation from atmospheric inputs (McNulty et al. 1991, Aber et al. 1998).

Potassium. While only marginally significant, resistant species did have lower K concentrations at the

Table 5. Regional correlations

A. Pairwise correlations variable	Foliar concentration					
	Ca	K	Mg	Mn	P	N
Percent infestation	0.18	0.24	0.09	0.35	0.35	0.26
Decline rating	0.34	0.22	0.03	0.34	0.46	0.11
Percent transparency	−0.04	0.38	−0.25	0.11	0.43	0.20
Percent dieback	0.02	0.31	−0.08	−0.03	0.34	0.17
Percent new growth	−0.70	0.21	−0.36	−0.56	−0.11	0.09
Percent live crown	0.03	−0.19	0.11	−0.07	−0.37	−0.07
B. Partial correlations variable	Ca	K	Mg	Mn	P	N
Percent infestation	0.23	0.10		0.02	0.26	0.30
Decline rating	0.42	0.30		0.11	0.47	
Percent transparency		0.20	−0.20		0.38	0.07
Percent dieback		0.20			0.27	0.08
Percent new growth	−0.43	−0.06	−0.30	−0.44		
Percent live crown					−0.36	

Correlations between foliar chemistry, infestation and decline variables in A show that multiple elements are significantly correlated with response variables. However, the partial correlations in B highlight only significant correlations while removing the effects of multicollinearity. Partial correlations highlight N as most strongly correlated with infestation densities, and Ca and P most strongly correlated with decline across the northeast.

Table 6.   Colonization correlations

	Ca	K	Mg	Mn	N	P
Number of live sistens present						
Preinfestation	−0.33 (0.19)	0.07 (0.80)	0.07 (0.78)	0.09 (0.74)	0.14 (0.57)	0.08 (0.76)
Postinfestation	0.03 (0.93)	0.49 (0.07)	−0.17 (0.55)	0.38 (0.16)	0.00 (0.99)	−0.54 (0.04)

Spearman's rho rank correlations between foliar chemistry and colonization success (with P values in parentheses) show that the number of live sistens present at the end of the study was strongly correlated with K and P concentrations at the time of collection.

Arnold Arboretum (Table 4). Of the 18 trees selected for the colonization study, 9 were successfully infested, including the resistant *T. chinensis* (3), *T. diversifolia* (4), and *T. heterophylla* (1). The numbers of live sistens present after 6 mo was positively correlated with initial K concentrations ($P < 0.10$; Table 6), and in combination with P, was able to predict the number of live sistens present across all species with $r^2 = 0.53$ and RMSE = 46 (Fig. 1), regardless of host species. K was also positively correlated with *A. tsugae* infestation levels and hemlock decline symptoms across the northeast (Table 5). These results suggest that, similar to N, K may have a palatability-based relationship with *A. tsugae*.

Results from the literature are mixed for K, indicating that the relationship between the element and resistance may be host/insect specific. Some studies concerning various insect herbivores find no appreciable effect of K on overall insect success (Elden and Kenworthy 1995, Kairo and Murphy 1999) Others have found that increased K imparts resistance to aphid species (Rohilla et al. 1993). Still others have found that there seems to be an increase in K concentration from primary producer to consumers, highlighting the importance of K in herbivore nutrition (Risley 1990). Our data suggest that, similar to N, K may be a limiting factor to *A. tsugae* population growth and warrants further study.

Phosphorus. Comparisons of the foliar chemistry databases suggest that resistant western species growing within their native range have significantly higher P concentrations than the susceptible eastern hemlock (Table 4). P was also the strongest correlate with the number of live sistens present at the end of the colonization study, with higher concentrations associated with low colonization success ($P < 0.10$; Table 6). Analysis of variance (ANOVA) indicated that colonization success was not significantly different between host species ($P = 0.39$) or assumed species resistance ($P = 0.97$). Instead chemistry was the primary factor in colonization success. Using only K and P, we were able to predict the number of live sistens present across all species with $r^2 = 0.53$ and RMSE = 46 (Fig. 1). These results suggest that P has a potential negative effect on *A. tsugae*.

Contrary to the colonization results, higher concentrations of P were positively associated with higher infestation percentages and increasing decline symptoms across the northeast (Table 5). However, it is possible that P concentrations in eastern hemlock have not reached a level necessary to impact *A. tsugae*. This seems plausible when comparing the average P concentration of 3,270 for resistant Asian species (Table 4) to 1,786 for infested eastern hemlock (Table 5).

Previous research backs up a resistance-enhancing hypothesis for P. Applications of P fertilizers have abated the effects of *Alphis craccivora* (Roch) on cowpea plants (Annan et al. 1997). Phosphorus was also identified as one of the factors contributing to aphid resistance in *Brassicae* crops (Chhillar and Verma 1985, Rohilla et al. 1993, Harada et al. 1996). Specifically, Marzo et al. (1997) found a close correlation between different P application rates and phytic acid levels in various pea varieties and a linear correlation between phytic acid content and *Bruchus pisorum* infestation. They concluded that greater phytate content could reduce the risk of *Bruchus* infestation in pea seeds. This research, combined with the data presented here, suggests that locations with high supplies of P may impart some degree of resistance to eastern hemlock.

Calcium. Foliar Ca was significantly higher in *T. sieboldii* (mean = 7,612 μmol) compared with eastern hemlock (mean = 1,989 μmol; t-test = −11.52, df = 4, $P = 0.003$). However, this effect was lost when combining species into resistant and susceptible groupings for analyses (Table 4). Similarly, preinfestation Ca concentration was negatively correlated with colonization success with only marginal significance (Table 6). To be consistent with these results, the positive correlation witnessed between *A. tsugae* infestation levels and
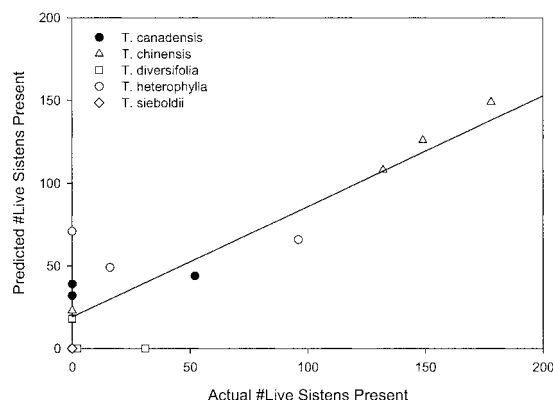
Fig. 1. Using only postinfestation K and P concentrations, the number of live sistens present at the end of the colonization study could be predicted with an $R^2 = 0.53$ and RMSE = 46. This relationship held across all species, with resistant and susceptible characteristics.

Ca across the northeast (Table 5) would have to show increased levels as a response to infestation. This is consistent with previous research, which found that aphid resistant tobacco species had Ca concentrations 10–100 times higher than susceptible species (Harada et al. 1996). Further study isolated exudates of $CaCl_2$ as having anti-aphid activity and named $CaCl_2$ as the driving factor in tobacco resistance to green peach aphids (*Myzus persicae*). They concluded that $CaCl_2$ is toxic to aphids rather than simply a feeding deterrent. Chhillar and Verma (1985) also identified Ca as contributing toward aphid resistance in barley plants. The culmination of evidence and results reported in the literature suggest that higher levels of Ca may increase resistance to *A. tsugae* by limiting population levels.

Ca was also positively correlated with hemlock decline across the regional study (Table 5). However, it is likely that this association with decline is a result of the close correlation between the percent new growth (a factor in decline calculations) and foliar Ca. As foliage ages, Ca oxalate accumulates in the foliage as a means to neutralize the oxalic acid byproducts of photosynthesis. Therefore, the close association between hemlock decline and Ca concentrations is most likely a result of differences between new and old growth foliage and their proportions in healthy versus declining trees.

Predictive Models. To estimate how much of the variability in infestation levels and hemlock decline can be attributed to foliar chemistry, multiple stepwise linear regressions were applied using chemistry only, landscape only, and full data models and predicted on an independent validation set to assess accuracy.

With $r^2 = 0.31$ and RMSE $= 22.72$, percent infestation was predicted by N, K, Ca, and P within 10% with 94% accuracy (Eq. 1; Fig. 2A). A hemlock decline regression built only on Ca, K, and P reported an r^2 of 0.43 and RMSE $= 0.85$, with a one-class tolerance accuracy of 93% (Eq. 2; Fig. 2B).

$$\%\text{Infestation} = -117 + 0.003\text{Ca} + 0.003\text{K}$$
$$- 0.001\text{CaK} + 0.01\text{P} + 51.3\text{N} - 0.09\text{PN} \quad [1]$$

$$\text{Decline} =$$
$$-1.6 + 0.001\text{Ca} + 0.001\text{K} - 4.8\text{CaK} + 0.001\text{P} \quad [2]$$

Previous research has primarily implicated landscape characteristics in patterns of hemlock decline (Royle and Lathrop 1999, Mayer et al. 2002, Orwig et al. 2002). Similar to this work, we found that several site factors were significantly associated with hemlock decline and could be used to predict decline across the northeast. This five-term model (Eq. 3 showed more severe decline symptoms with higher infestation levels (I), drier than normal growing season (Palme Drought Index D), higher hemlock stocking (H), southern and western exposures (A), and ridge top/side-slope positions (L), producing an $r^2 = 0.37$ and RMSE $= 0.90$ (Eq. 3, Fig. 3A), with a one-class tolerance accuracy of 90%. This is all in agreement with previous work indicating that water availability may be a stressor, speeding decline in the drought sensitive eastern hem-
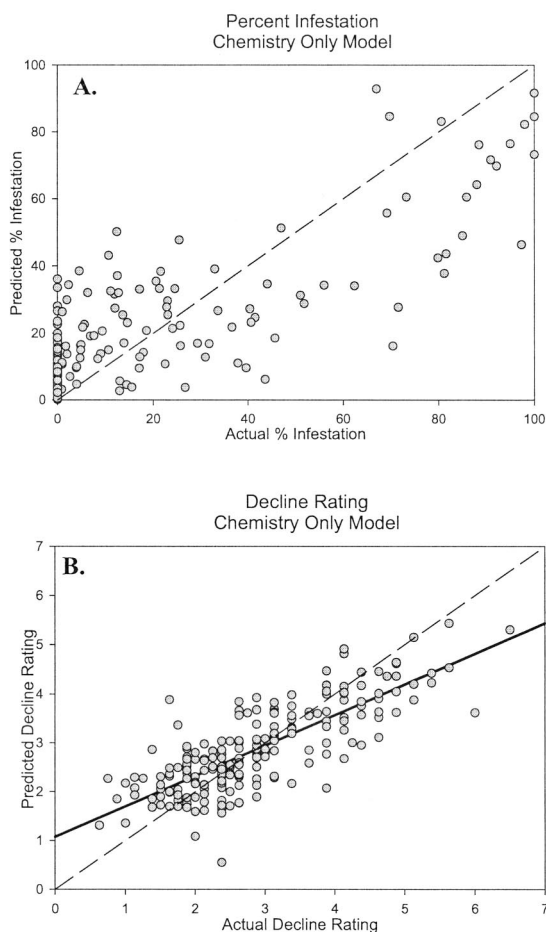

Fig. 2. Linear regression models to predict HWA infestation levels (**A**) and hemlock decline (**B**) exemplify how much of the variability can be explained using foliar chemistry. Based only on N, K, P, and Ca, infestation (Eq. 1, decline, and Eq. 2 were predicted on an independent validation set with $r^2 = 0.31$, 0.43 and RMSE $= 22.72$, 0.85, respectively).

lock (Bonneau et al. 1997, Orwig and Foster 1999, Young et al. 1999, Orwig et al. 2002).

$$\text{Decline} = -1.29 + 0.01\text{I} + 0.31\text{D} + 0.03\text{H} - 0.17\text{A}$$
$$- 0.36\text{L (flat} - \text{ridge)} - 0.42\text{L (flat} - \text{bench)} \quad [3]$$

The limitation is that, with an $r^2 = 0.37$, even including *A. tsugae* infestation levels (the strongest correlate with health), a landscape characteristics only–based model is only able to account for a little over one third of the variability in decline (Fig. 3A). The fact that the four-term chemistry–only model (Eq. 2) can account for more of the variability in decline highlights the importance of chemistry in *A. tsugae*/hemlock infestation and decline patterns (Fig. 2B).

When we allow the model to select from all potential variables, the same landscape variables are retained (Years infested Y, Palmer Drought Index D, Basal area B, Aspect A, and Landscape Position L)
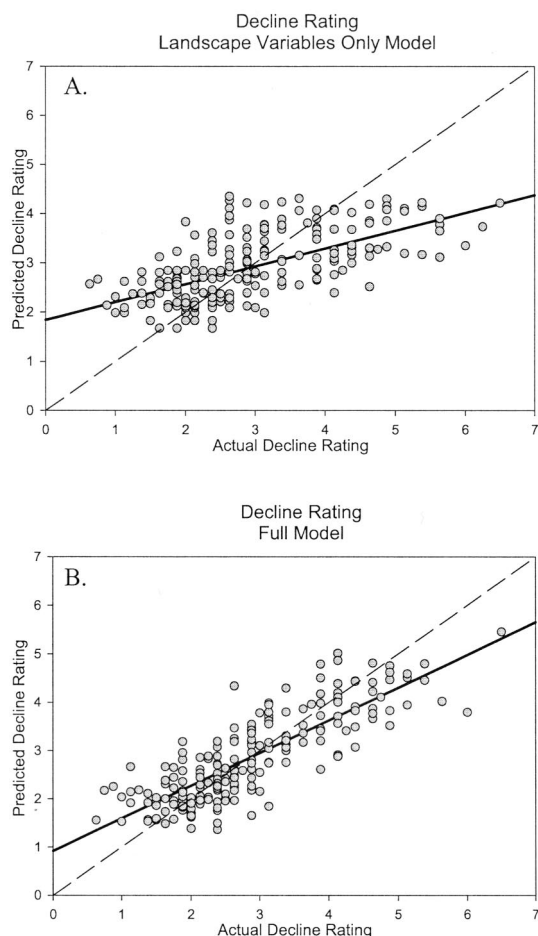
Decline Rating
Landscape Variables Only Model

Decline Rating
Full Model

Fig. 3. A stepwise linear regression model based only on significant landscape characteristics (A) was able to predict hemlock decline with an $r^2 = 0.37$, RMSE = 0.90, and a one-class tolerance accuracy of 90% (Eq. 3); by allowing the addition of significant chemistry variables (B), the model is improved to $r^2 = 0.68$, RMSE = 0.64, and 98% one-class tolerance accuracy (Eq. 4).

with the addition of Ca, P, and N, resulting in $r^2 = 0.68$, RMSE = 0.64, and 98% one-class tolerance accuracy (Eq. 4; Fig. 3B). This final model is able to account for over two thirds of the variability in hemlock decline across the northeast. This indicates that the addition of chemistry data should greatly improve the predictive ability of *A. tsugae* susceptibility models.

$$Decline = -1.09 + 0.1Y + 0.0001Ca + 0.001P - 0.13N$$

$$+ 0.36D + 0.0003PD + 0.005B + 0.0001CaB - 0.001A$$

$$+ 0.0001PA - 0.08L + 13.16LN \quad [4]$$

We concur that other factors not measured here are necessary to more fully explain the variability in hemlock decline rates after *A. tsugae* infestation. It is likely that the inclusion of other organic compounds, such as phenolics or other defensive chemicals, would further improve this model. It is also likely that genetics plays a large role and should be examined to help isolate the mechanisms of host resistance to *A. tsugae*. However, the ease of assessing foliar chemistry (particularly N, K, P, and Ca) provides a useful and necessary addition to any hemlock susceptibility model.

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